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PCT

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>5</sup> :</b> <b>A61K 39/395, 47/48, 39/00</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 90/11779</b> <b>(43) International Publication Date:</b> 18 October 1990 (18.10.90)
<b>(21) International Application Number:</b> PCT/GB90/00476 <b>(22) International Filing Date:</b> 30 March 1990 (30.03.90) <b>(30) Priority data:</b> 8907310.0 31 March 1989 (31.03.89) GB <b>(71) Applicant (for all designated States except US):</b> MEDICAL RESEARCH COUNCIL [GB/GB]; 20 Park Crescent, London W1N 4AL (GB). <b>(72) Inventor; and</b> <b>(75) Inventor/Applicant (for US only) :</b> LACHMANN, Peter, Julius [GB/GB]; Molecular Immunopathology Unit, Medical Research Council Centre, Hills Road, Cambridge CB2 2QH (GB). <b>(74) Agents:</b> CRESSWELL, Thomas, Anthony et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5EU (GB).		<b>(81) Designated States:</b> AT (European patent), BE (European patent), CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> HETEROCONJUGATES  <b>(57) Abstract</b>  An antigen-antibody conjugate for treatment of a tumour in a patient wherein the antigen is an antigen against which the patient has immunity and the antibody is an antibody capable of specifically binding the cells of the tumour.		

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HETEROCONJUGATES

The present invention relates to novel targetting agents and to their use in the treatment of cancers.

Radio- and chemotherapy are now well established as  
5 cancer treatments, as is surgery, for clearly defined  
tumours. However these techniques are less effective at  
destroying small lesions due to metastasis and, with  
surgery, there is always a risk of leaving behind small  
areas of cancerous tissue. The present invention is  
10 particularly concerned with providing a means to clear up  
such small pockets of cancerous cells and is therefore  
considered mainly as an adjunct to the already established  
therapeutic and surgical techniques. In particular, the  
invention aims to recruit aspects of the patient's own  
15 immune system and to target this against the tumour cells.

Accordingly the present invention, in one aspect  
provides a method for the treatment of the human or animal  
body comprising administering an effective, non-toxic  
amount of a targetting agent which is a conjugate of an  
20 antigen and an antibody, or fragments thereof, the antigen  
being selected such that the patient already has immunity  
to the antigen and the antibody being selected to bind  
specifically to the tumour cells.

Figs. 1a & 1b show the clone 3-PPD conjugate mediated  
25 lysis of C3 coated MC6A tumour cells in cytotoxicity  
assays.

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Figs. 2a & 2b show the MM2-9B6-PPD conjugate mediated lysis of B16-F10 tumour cells in cytotoxicity assays.

Figs. 3a & 3b show the tumour cytostasis mediated by clone supernatant.

5 Figs. 4a & 4b show lymphokine assays.

The antigen may be any antigen to which the patient has previously been exposed, or any antigen which cross-reacts with lymphocytes in the patient's blood. Examples of suitable antigens include those of the childhood illnesses  
10 such as measles, chickenpox or mumps and other antigens to which the population in general is likely to have induced immunity, for instance, tetanus, typhoid and tuberculosis. The latter is particularly relevant to the present invention as the vast majority of the population have been  
15 immunised using BCG vaccine against Mycoplasma tuberculosis; the cross-reacting PPD (purified protein derivative) from M. tuberculosis may be used in the present method and is particularly preferred because of the very strong immune reaction which it elicits.

20 Fragments of such antigens may also be used in the invention provided that they retain the epitope which will be recognised by the patient's immune system. In the case of PPD, which consists of a number of different polypeptide sub-units, any antigenic sub-unit or indeed any antigenic  
25 domain of one of the sub-units, may be used as the antigen.

The antibody used in the conjugate may be any antibody

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or fragment thereof which retains an antigen-binding site, such as the Fab' fragment, which will bind specifically to the tumour cells to be destroyed. Many tumour specific antigens are now known and others will be discovered in the future; antibodies, whether polyclonal or monoclonal (the latter are preferred) against such antigens may be used in the present invention. Certain tumours do not express tumour-specific antigens but these may be targetted using antibodies against neo-antigens in bound components of complement, such as C3 which are often found to accumulate on the surface of those tumour cells which activate the alternative complement pathway. Antibodies against C3 or other complement components may also be used when the conjugates of the invention are to be administered after conventional monoclonal antibody treatment of cancers. (Such treatment, using tumour specific antibodies, results in the tumour cells becoming covered first in the anti-tumour antibodies and then in complement which binds to the anti-tumour antibodies). When anti-complement antibodies are used it is preferred that they are directed against neo-antigenic sites, i.e, sites which are only formed or exposed once complement binding has occurred, in order that binding to unbound complement is avoided.

In addition to being specific for the tumour cells to be destroyed, the antibodies should preferably be derived from antibody-producing cells of the same species as the patient

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or should be modified to mask or remove any species-specific determinants other than those of the same species as the patient.

The antigen (or fragment thereof) and antibody (or  
5 fragment thereof) may be coupled by any conventional method for covalently binding such materials. For instance, linking groups may be bound to the antigen and to the antibody and the linking groups are then coupled together. Typically one linking group is formed using the reagent  
10 SMCC (succinimidyl4-(N-maleimidomethyl)cyclohexane-1-carboxylate described in Yoshitake, S. et al (1979) Eur. J. Biochem, 101, 395-399 and Mahan, D.G. et al (1987) Anal. Biochem, 162, 163-170) and the other linking group is formed using SPDP (N-succinimidyl-3-(2-pyridyldithio)  
15 propionate described in Walden, P. et al (1986) J. Mol. Cell. Immunol. 2, 191-197 and Gordon, R.D. et al (1987) Proc. Natl. Acad. Sci. 84, 308-312), both available commercially from Pierce U.K. Ltd, and the two linking groups are then coupled, or conjugated, by the formation of  
20 thiomaleimide bonds.

Coupling techniques should be selected so as to avoid impairing either the antigenicity of the antigen or the affinity of the antibody for the tumour cells; where necessary the product may be fractionated to obtain  
25 quantities of effective conjugate.

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The present invention also provides a conjugate comprising an antigen and an antibody, or fragments thereof, covalently coupled via linking groups. Processes for coupling the antigen and antibody to form such a  
5 conjugate form a further aspect of the invention.

The invention further provides such conjugates for use in a therapeutic method for the treatment of the human or animal body and the use of such conjugates in the manufacture of a medicament for use in the treatment of  
10 cancer.

The conjugates of the invention may be administered as such but are preferably administered as pharmaceutical compositions also comprising a pharmaceutically acceptable diluent or carrier. Typical diluents and carriers include  
15 water for injection and other injection media.

Compositions may be presented in unit or multi-dosage form. For administration by injection the compositions may be presented in ready-to-use form or as a concentrate or dry powder for reconstitution, e.g. using water for injection,  
20 prior to use. The compositions will generally be sterile and pyrogen free. The compositions may also comprise accessory ingredients such as antibacterial and antifungal agents, buffers, salts, agents to adjust the tonicity of the composition, anti-oxidants, wetting agents and  
25 suspending agents to improve the solubilisation or suspension of the conjugates and analgesics or anaesthetics



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to reduce pain at the injection site. These compositions form a further aspect of the invention.

The conjugates and compositions of the invention will usually be administered by injection, preferably by the  
5 intravenous route, or by infusion. Where appropriate, injection or infusion directly into a tumour or lesion is also contemplated.

The dosage amounts will be depend on the patient- for instance body weight, age, sex and general state of health  
10 - the size, location and nature of the tumour and the rate of clearance of the agent as well as the antigenicity of the conjugate and the level of the patient's immunity to the antigen or fragment thereof. As a guide the dosage of a conjugate of PPD and an antibody would be in the range of  
15 from 10 to 50 $\mu$ g PPD per injection.

Dosage regimes also depend upon a number of factors including those outlined above. For infusion, the administration may take place over the period of several hours, possibly repeated daily, or may continue  
20 uninterrupted for days. When given by injection, the conjugates are preferably administered either daily or at longer intervals, for instance of a few days. Administration may be repeated as necessary.

Without wishing to be bound by theory, it is believed  
25 that the conjugates of the invention will bind to the tumour cells by virtue of the interaction between the

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antibody part of the conjugate and the corresponding tumour specific antigen or complement component. This results in the tumour cells being labelled with the antigen part of the conjugate. This is then recognised by the cellular  
5 immune system of the patient leading to the development of a local delayed-type hypersensitivity (DTH) reaction with destructive effects against tumour cells, and possibly adjacent cells, effected by T helper cells and their secreted products. The generation of cytotoxic T cells  
10 should also be enhanced as a result.

It is therefore important that the patient has T-cell immunity against the antigen to be used in the conjugate. Treatment involving the use of conjugates according to the invention may therefore involve testing samples of tissue  
15 or body fluid from the patient to identify a suitable antigen against which the patient has T-cell immunity and selection of a conjugate of such an antigen.

The invention will now be illustrated by the following Example:

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EXAMPLE 1Preparation of PPD-monoclonal antibody (MAb)  
heteroconjugates.

MAbs were used which were specific for a tumour  
5 associated antigen of CS7/BL6 melanomas, or for the human  
complement component C3d, which was fixed de novo to the  
surface of tumour cells. The ability of the conjugate to  
induce PPD-specific T-cell activation, lymphokine secretion  
and tumour cell cytolysis/cytostatis in vivo was determined  
10 with a view to focusing a DTH response against selected  
tumour target in BCG immunized animals.

Materials used

Effectors: Synergistic non-adherent spleen cells from  
BCG immunized mice or a L3T4+, Lyt-, PPD reactive T-cell  
15 clone (PPD-MW1).

Monoclonal antibodies and tumour lines:

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<u>IgG MAb</u>	<u>Specificity</u>	<u>Tumour Target</u>
Clone 3	Human C3d	Fibrosarcoma MC6A (MC6A cells coated with C3 via the classical pathway)
5		
MM2-9B6	C57/BL6 melanomas	B16-F10

Methods usedMAb-PPD Conjugation

- 10 MAb was covalently linked to PPD using the hetero-bifunctional cross linkers SPDP and SMCC.

Cytotoxicity Assay

- Tumour cells were pretreated (45 mins, 4°C) with optimal concentrations of either MM2-9B6-PPD or Clone 3-PPD
- 15 conjugates. Control cells were treated with equivalent concentrations of the MAbs or PPD alone. Tumour cells were then co-cultured with effectors for 16 hrs. at various E.T.

- 10 -

ratios. Specific cytotoxicity was determined using a standard  $^{51}\text{Cr}$  release assay.

#### Cytostasis Assay

Serially diluted supernatant from PPD activated clone  
5 PPD-MW1 was added to both tumour cell lines and cytostasis measured by the inhibition of  $^3\text{H}$  TdR incorporation on day 3.

#### Lymphokine Assays

##### Tumour necrosis factor (TNF) alpha/beta

10 Clone PPD-MW1 was activated by B16-F10 cells pretreated with MM2-9B6-PPD and TNF production determined using the TNF sensitive cell line L929.  $^{51}\text{Cr}$  labelled L929 cells were incubated with serially diluted control and activated clone supernatant for 16 hrs in the presence of actinomycin D.

15 Susceptibility of the tumour targets to human recombinant TNF (rTNF) alpha was measured using a 16 hr  $^{15}\text{Cr}$  release assay, and by inhibition of  $^3\text{H}$  TdR incorporation on day 3.

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Macrophage activation Factor (MAF)

Serially diluted supernatant from PPD activated clone PPD-MW1 was added to purified peritoneal macrophages in the presence of LPS for 18 hrs. Macrophage activation was  
5 determined by lysis of  $^{51}\text{Cr}$  labelled P815 cells following an 18 hr co-culture.

Tumour Growth In vivo

Control and MM2-9B6-PPD conjugate treated B16-F10 cells were injected SC into BCG immunized or normal C57/BL6 mice  
10 ( $3 \times 10^5$  cells/animal). Tumours were excised on day 11 and weighed. Significance levels were determined using the Mann-Whitney U test.

Cytotoxicity assays

Clone 3-PPD conjugate mediated significant levels of  
15 cytotoxicity against C3 coated MC6A tumour cells using both immune spleen cells (Fig. 1a) and the clone (Fig. 1b) at high E:T ratios. MAb or PPD alone did not increase cytotoxicity.

MM2-9B6-PPD conjugate failed to mediate significant  
20 levels of cytotoxicity against B16-F10 tumour cells when immune spleen cells were used as a source of effectors. (Fig

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2a). Marginal cytotoxicity above control levels was, however, evident when the PPD-reactive clone was used.

#### Cytostasis assay

The supernatant from PPD activated clone PPD-MW1 was  
5 able to induce high levels of cytostasis in both tumour cell lines MC6A and B16-F10 (Figs 3a & 3b). This cytostasis could not be attributed solely to the action of rTNF alpha.

#### Lymphokine assays

10 Tumour cells pretreated with MM2-9B6-PPD conjugate were able to stimulate clone PPD-MW1 to produce significant levels of TNF, alpha/beta (Fig 4a). Significant MAF activity at a supernatant titer of 1/32 was also found. Susceptibility of the tumour lines of rTNF alpha (Fig. 4b)  
15 correlated well with the levels of cytotoxicity observed in vitro with PPD-reactive T-cells (Figs. 1 & 2).

#### Tumour growth inhibition in vivo

Pretreatment of B16-F10 tumour cells with MM2-9B6-PPD conjugate, but not PPD or the MAb alone, resulted in  
20 significant tumour growth inhibition in BCG immunised animals.

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<u>Treatment</u>	<u>Non-Immune (g)</u>	<u>BCG Immune (g)</u>
PPD	2.8 $\pm$ 0.7*	2.2 $\pm$ 0.6
MAb	2.7 $\pm$ 0.8	1.7 $\pm$ 0.9
MAb-PPD	1.5 $\pm$ 1.0	0.9 $\pm$ 0.8**

5 \* Mean wet weight  $\pm$  1SD (n=10)

\*\* Significantly different from control groups (p<0.05).

#### Experimental Conclusion

MAb-PPD heteroconjugates specific for a tumour associated antigen, or a de novo fixed complement  
10 component, can be used to focus PPD-specific T-cells onto tumour targets in vitro.

Heteroconjugate treated tumour cell targets activate PPD-specific T-cell clones, resulting in concomitant release of significant levels of TNF alpha/beta and MAF.  
15 Susceptibility of the tumour targets to rTNF alpha correlated well with the levels of cytotoxicity achieved over 18 hours in vitro with PPD-specific T-cells. The high levels of cytostatis achieved over 72 hours could not, however, be attributed solely to the activity of rTNF alpha  
20 and may reflect synergy between the TNF alpha/beta and MAF.

The activation of PPD-specific T-helper cells at sites of tumour growth in vivo may result in the recruitment of



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other tumourcidal effects, and ultimately induce a DTH response. Heteroconjugates directed to complement components should further allow the activation of T-cells at sites of complement fixation, and this may be exploited  
5 to enhance the effectiveness of conventional MAb therapy. MM2-9B6-PPD conjugate can give a significant reduction in the growth of B16-F10 cells in BCG immunised animals.

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CLAIMS

1. An antigen-antibody conjugate for treatment of a tumour in a patient  
wherein the antigen is an antigen against which the patient has immunity  
and the antibody is an antibody capable of specifically binding the cells of the tumour.
2. A conjugate according to claim 1 wherein the antigen is an antigen to which the patient has previously been exposed, an antigen which is capable of cross-reacting with lymphocytes in the patient's blood or a fragment of such an antigen containing an epitope which will be recognised by the patient's immune system.
3. A conjugate according to claim 2 wherein the antigen is a measles, chickenpox, mumps, tetanus, typhoid or tuberculosis antigen.
4. A conjugate according to claim 3 wherein the antigen is the cross-reacting purified protein derivative (PPD) of Mycoplasma tuberculosis or an antigenic sub-unit or antigenic domain of a sub-unit of PPD.
5. A conjugate according to any one of claims 1 to 4 wherein the antibody is an antibody which binds specifically to a tumour specific antigen or an antibody which binds specifically to a neo-antigenic site of a bound complement component, or a fragment of such an antibody containing an antigen-binding site.

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6. A conjugate according to claim 5 wherein the antibody is an antibody against a tumour specific antigen or a neo-antigenic site of bound C3 or a Fab' fragment of such an antibody.

7. A conjugate according to any one of claims 1 to 6 wherein the antibody is a polyclonal antibody.

8. A conjugate according to any one of claims 1 to 6 wherein the antibody is a monoclonal antibody produced by a cell derived from an antibody-secreting cell of the same species as the patient or which has been modified to mask or remove species-specific determinants of species other than those of the same species as the patient.

9. A conjugate according to any one of claims 1 to 8 wherein the antibody and antigen are coupled by the linked residues of a linking group on the antibody and a different linking group on the antigen.

10. A conjugated according to claim 9 wherein the antibody and antigen are coupled by the thiomaleimide-linked residues of succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate and N-succinimidyl-3-(2-pyridylthio)propionate.

11. A conjugate according to any one of claims 1 to 10 for use in a method of treatment of the human or animal body.

12. A conjugate according to any one of claims 1 to 10 for use in a method for the therapeutic treatment of a

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pati nt having a tumour.

13. Use of a conjugate according to any one of claims 1 to 10 in the manufacture of a medicament for use in the treatment of cancer.

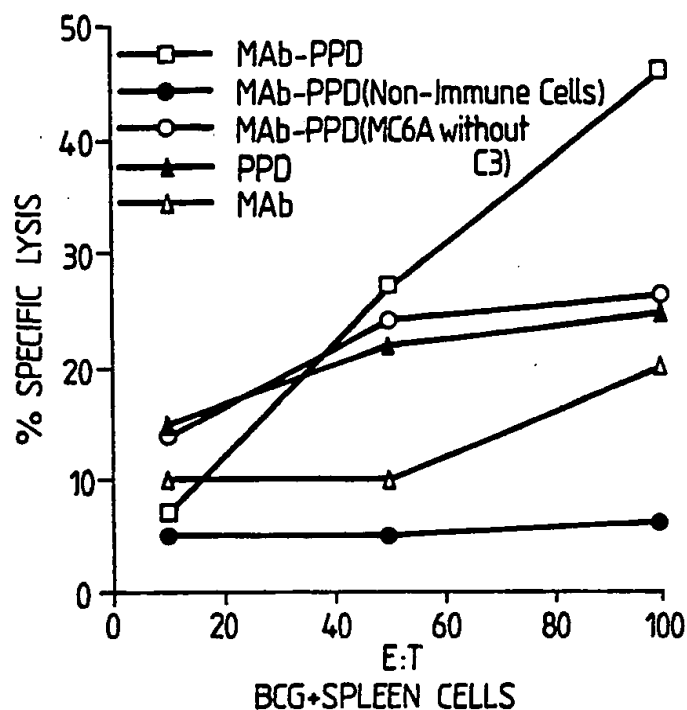
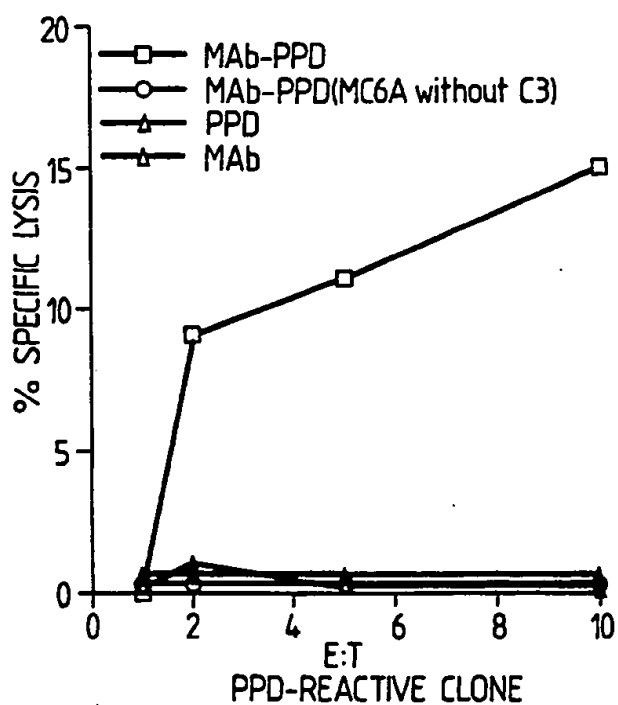
14. A method for the treatment of the human or animal body comprising administering an effective, non-toxic amount of a targeting agent which is a conjugate of an antigen and an antibody, or fragments thereof, the antigen being selected such that the patient already has immunity to the antigen and the antibody being selected to bind specifically to the tumour cells.

15. A method according to claim 14 comprising the use of a conjugate according to any one of claims 1 to 12 or a medicament produced according to claim 13.

16. A process for producing a conjugate comprising coupling an antigen against which a patient has immunity and an antibody capable of specifically binding to cells of a tumour.

17. A process according to claim 16 for producing a conjugate according to any one of claims 1 to 12.

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*Fig.1a.**Fig.1b.*

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Fig.2a.

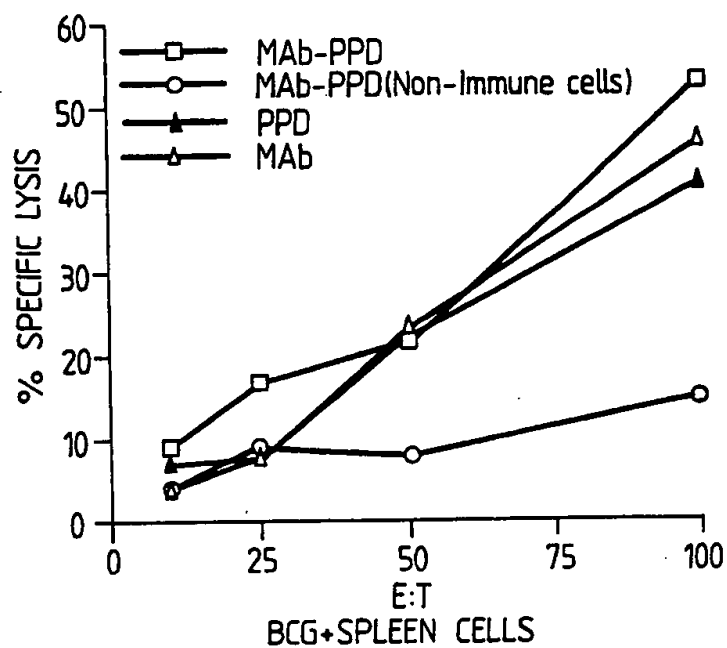
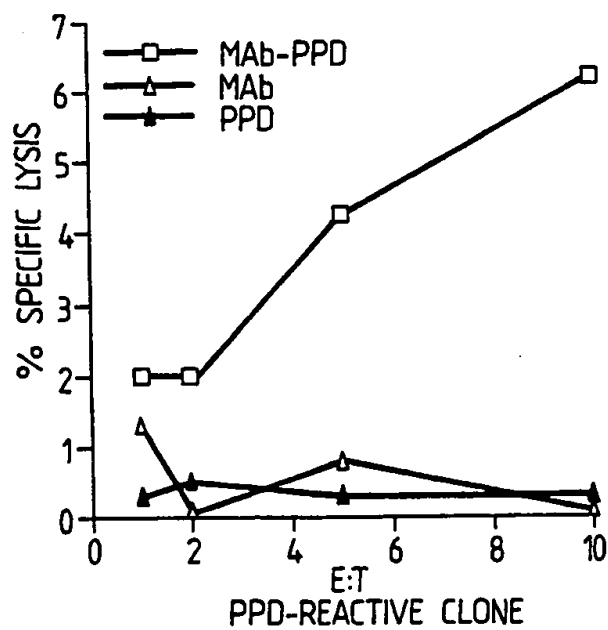


Fig.2b.



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Fig.3a.

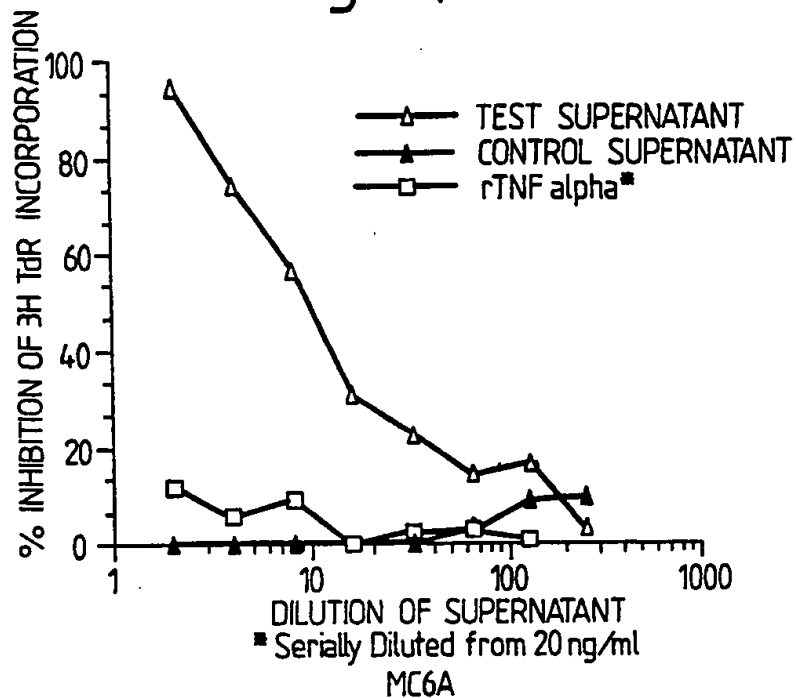
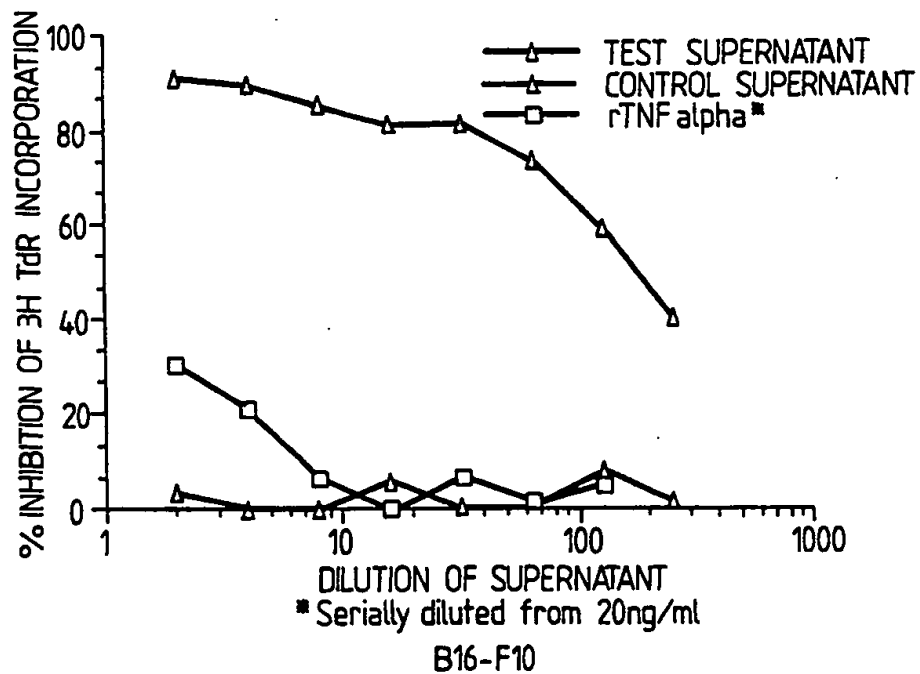


Fig.3b.



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Fig.4a.

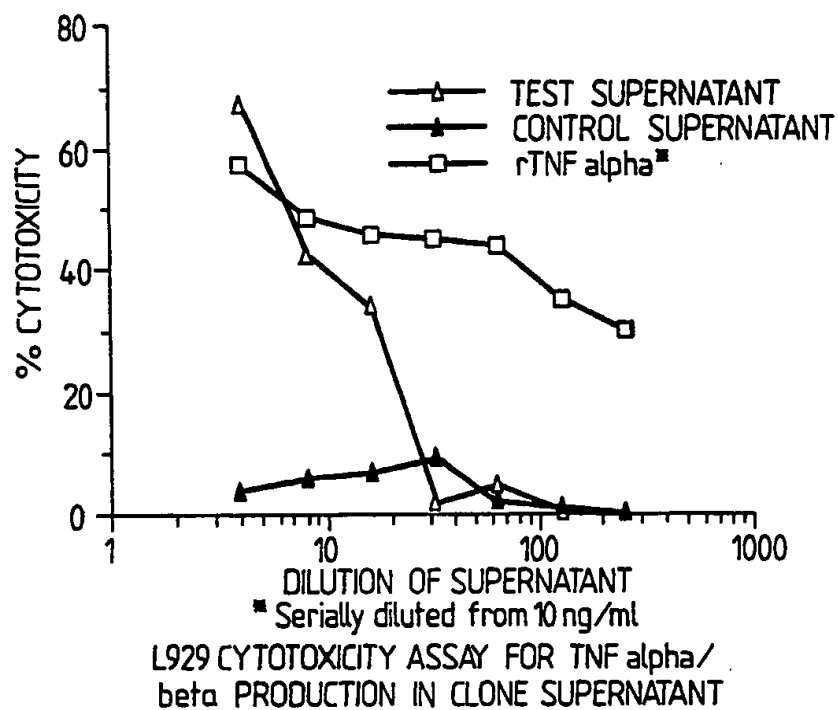
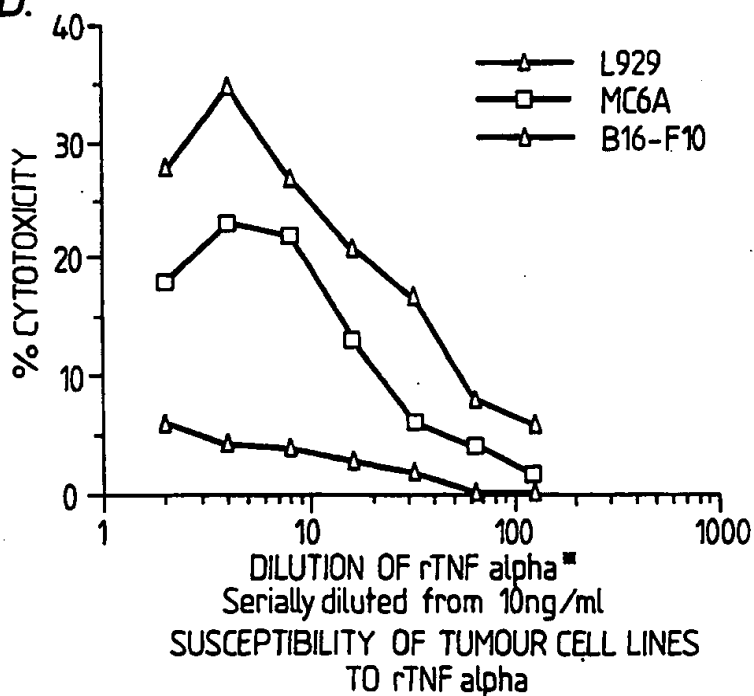


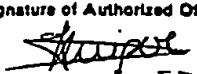
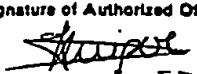
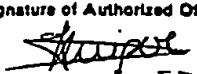
Fig.4b.





# INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/00476

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>4</sup> According to International Patent Classification (IPC) or to both National Classification and IPC IPC <sup>5</sup> : A 61 K 39/395, 47/48, 39/00																	
<b>II. FIELDS SEARCHED</b> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Minimum Documentation Searched <sup>7</sup></div> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border-bottom: 1px solid black;">Classification System <sup>1</sup></td> <td style="width: 50%; border-bottom: 1px solid black;">Classification Symbols</td> </tr> <tr> <td style="padding: 5px;">IPC<sup>5</sup></td> <td style="padding: 5px;">A 61 K</td> </tr> </table> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black;">Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched <sup>8</sup></div>			Classification System <sup>1</sup>	Classification Symbols	IPC <sup>5</sup>	A 61 K											
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<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b> <table style="width: 100%; border-collapse: collapse;"> <tr> <th style="width: 10%; border-bottom: 1px solid black;">Category <sup>6</sup></th> <th style="width: 70%; border-bottom: 1px solid black;">Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup></th> <th style="width: 20%; border-bottom: 1px solid black;">Relevant to Claim No. <sup>13</sup></th> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">EP, A, 0245078 (CONNAUGHT LABORATORIES LIMITED) 11 November 1987 see page 3, line 11 - page 4, line 4 --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-4</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y</td> <td style="padding: 5px;">Experientia, volume XVIII, no. 12, 15 December 1962, L. Forró et al.: "A new type of antigen induced by chemical linkage of Mycobacterium tuberculosis and y-Globulin", pages 553-554 see the whole article --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-4</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y,P</td> <td style="padding: 5px;">EP, A, 0336405 (TAKEBA CHEMICAL INDUSTRIES) 11 October 1989 see page 1, line 42 - page 6, line 43 --</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-13,16,17</td> </tr> <tr> <td style="text-align: center; vertical-align: top; padding: 5px;">Y,P</td> <td style="padding: 5px;">EP, A, 0324625 (BUNGE (AUSTRALIA) PROPRIETARY LIMITED) ./.</td> <td style="text-align: center; vertical-align: top; padding: 5px;">1-13,16,17</td> </tr> </table>			Category <sup>6</sup>	Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>	Y	EP, A, 0245078 (CONNAUGHT LABORATORIES LIMITED) 11 November 1987 see page 3, line 11 - page 4, line 4 --	1-4	Y	Experientia, volume XVIII, no. 12, 15 December 1962, L. Forró et al.: "A new type of antigen induced by chemical linkage of Mycobacterium tuberculosis and y-Globulin", pages 553-554 see the whole article --	1-4	Y,P	EP, A, 0336405 (TAKEBA CHEMICAL INDUSTRIES) 11 October 1989 see page 1, line 42 - page 6, line 43 --	1-13,16,17	Y,P	EP, A, 0324625 (BUNGE (AUSTRALIA) PROPRIETARY LIMITED) ./.	1-13,16,17
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Y,P	EP, A, 0336405 (TAKEBA CHEMICAL INDUSTRIES) 11 October 1989 see page 1, line 42 - page 6, line 43 --	1-13,16,17															
Y,P	EP, A, 0324625 (BUNGE (AUSTRALIA) PROPRIETARY LIMITED) ./.	1-13,16,17															
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>10</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"A" document member of the same patent family</p> </div> </div>																	
<b>IV. CERTIFICATION</b> <table style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">Date of the Actual Completion of the International Search</td> <td style="width: 50%; border-bottom: 1px solid black; padding: 5px;">Date of Mailing of this International Search Report</td> </tr> <tr> <td style="text-align: center; padding: 5px;">25th June 1990</td> <td style="text-align: center; padding: 5px;">18 JUL 1990</td> </tr> <tr> <td style="border-bottom: 1px solid black; padding: 5px;">International Searching Authority</td> <td style="border-bottom: 1px solid black; padding: 5px;">Signature of Authorized Officer</td> </tr> <tr> <td style="text-align: center; padding: 5px;">EUROPEAN PATENT OFFICE</td> <td style="text-align: center; padding: 5px;">   Mme N. KUIPER </td> </tr> </table>			Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	25th June 1990	18 JUL 1990	International Searching Authority	Signature of Authorized Officer	EUROPEAN PATENT OFFICE	 Mme N. KUIPER							
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## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

19 July 1989  
see page 1, line 28 - page 5, line 43

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V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE :

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 14, 15 because they relate to subject matter not required to be searched by this Authority, namely:

See PCT-Rule 39.1 (iv): methods for treatment of the human or animal body by surgery or therapy as well as diagnostic methods.

2. ☐ Claim numbers \_\_\_\_\_, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers \_\_\_\_\_, because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING :

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

## Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

GB 9000476  
SA 35807

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 13/07/90  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0245078	11-11-87	JP-A- 63045228	26-02-88
EP-A- 0336405	11-10-89	None	
EP-A- 0324625	19-07-89	None	